ON THE INTERACTION OF THERMOLABILE AND THERMOSTABLE OPSONIC SUBSTANCES IN NORMAL SERUM.*

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THAT opsonins normally present in serum were destroyed by heating to 60° for 10 minutes was ascertained by Wright and Douglas.[1] Hektoen and Ruediger[2] found that "opsonins are thermolabile substances of a constitution analogous to that of toxins and complements in that they seem to have two groups, haptophore and opsoniferous." Other observers, among them Levaditi,[3] Savtschenko,[4] Tarassevitch,[5] and Neufeld[6] and his coworkers, have found that opsonic substances in immune sera are largely thermostable, in that respect apparently differing from the sensitizing bodies in normal serum. Muir and Martin[7] were able to establish other differences in behavior between normal and immune opsonic substances and came to the conclusion that the thermolabile opsonin of normal serum and the thermostable opsonin of immune serum are distinct classes of substances, showing different combining relationships as well as different heat resistances.

In 1907 Dean[8] pointed out that on mixing appropriate dilutions of a heated immune serum with dilutions of normal fresh serum, phagocytic indices were obtained greater than resulted from the sum of the two acting separately. Later Cowie and Chapin[9] were able to show a similar phenomenon in the case of heated and diluted normal human serum with respect to phagocytosis of staphylococci. In

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[1] *Proc. Royal Soc.*, 1903, 72, p. 357; 1904, 73, p. 128.

[2] *Jour. Infect. Dis.*, 1905, 2, p. 128.

[3] *Ann. de l'Inst. Past.*, 1901, 15, p. 904.

[4] *Ibid.*, 1902, 16, p. 106.

[5] *Ibid.*, 1902, 16, p. 127.

[6] Neufeld and Töpler, *Centralbl. f. Bakt.*, 1907, 38, p. 456; Hüne, *Arb. a. d. kais. Gesundh.*, 1907, 25, p. 164.

[7] *Proc. Royal Soc.*, 1907, 79, p. 187.

[8] *Proc. Royal Soc.*, 1907, 79, p. 399; see also *ibid.*, 1905, 76, p. 506.

[9] *Jour. Med. Res.*, 1907, 17, pp. 95, 213.

the same year Neufeld and Bickel[1] found that "sublytic" amounts of serum containing hemolytic amboceptor, in the presence of minute quantities of normal serum, could so act on erythrocytes as to induce active phagocytosis.

The results in this article are based on observations along similar lines upon normal serum exclusively.

Except where otherwise indicated, an avirulent strain of pneumococcus was used throughout. It was kept up on blood-agar, suspensions for immediate use being prepared by growing for 24 hours in glucose-free broth; this method, worked out by Dr. Rosenow in this laboratory, has been found to yield very satisfactory suspensions. The technic otherwise was essentially that of Wright and Douglas.

At the outset, using only one specimen of human serum (Serum "E") and working with pneumococcus, I was unable to reproduce the results of the previous investigators. The following table (Table 1) serves to illustrate the character of the results repeatedly obtained with this serum. Diluted serum is normal serum ("E"), diluted 10 times in NaCl solution. Heated serum is "E" serum heated to 60° for 10 minutes. Leucocytes are human blood cream, washed. The figures give the average number of bacteria taken up per leucocyte, 50 leucocytes being counted; 100 if individual counts showed much variation.

TABLE 1.

Dil. serum	+	leucocytes	+	bact. susp.	+	NaCl sol.	=	1.9.
H't	"	+	"	+	"	"	+	"	"	= 1.0
"	"	+	"	+	"	"	+	dil. serum	=	2.6
Nor.	"	+	"	+	"	"	+	NaCl sol.	=	5.2

I then tried to substitute diluted guinea-pig serum for the diluted human serum. All factors are as in preceding experiments, except that dilute serum is guinea-pig serum diluted 10 times. Table 2 shows the results so obtained.

TABLE 2.

Heated serum	+	leucocytes	+	bact. susp.	+	NaCl sol.	=	1.4
G. P. ser. dil.	+	"	+	"	"	+	"	"	=	1.1
" "	+	"	+	"	"	+	h'd serum	=	2.8
Nor. human ser.	+	"	+	"	"	+	NaCl sol.	=	6.4
Nor. G. P.	"	+	"	+	"	"	+	"	"	= 3.8

We see that the use of guinea-pig serum diluted, in place of diluted human serum, did not promote phagocytosis by the heated serum.

[1] *Arb. a. d. kais. Gesundh.*, 1907, 27, 310.

Experiments were also instituted to determine whether or not variations in the dilution of the suspension of organisms could account for the results. Full, half, and quarter strength dilutions of the suspended organisms were used, with negative results. Inasmuch as the explanation was finally found in another direction, insertion of the results here is scarcely necessary.

At this point another human serum ("G") was used, all other factors being as in Table 1; the results obtained with this are given in Table 3.

TABLE 3.

"G"	serum	heated,	leucocytes,	bact.	susp.,	NaCl sol.	=0.7
"	"	diluted,	"	"	"	" "	=0.7
"	"	heated,	"	"	"	"G" ser. dil.	=2.6
"	"	normal,	"	"	"	NaCl sol.	=3.0

With this serum results apparently corroborating those of previous investigators were obtained.

The next experiment (Table 4), was made in an effort to differentiate the particular factor that prevented the promotion of phagocytosis in the case of the first serum (Serum "E"). Sera "E" and "G" and corpuscles "E" were used, all other factors as before.

TABLE 4.

"E"	ser.	h'd.	+	leucocytes	+	bact.	susp.	+	NaCl sol.	=0.6
"	"	dil.	+	"	+	"	"	+	" "	=0.4
"	"	"	+	"	+	"	"	+	"E" ser. h'd.	=0.9
"	"	nor.	+	"	+	"	"	+	NaCl sol.	=4.3
"G"	"	h'd.	+	"	+	"	"	+	" "	=1.0
"	"	dil.	+	"	+	"	"	+	" "	=0.5
"	"	"	+	"	+	"	"	+	"G" ser. h'd.	=4.0
"	"	nor.	+	"	+	"	"	+	NaCl sol.	=3.9
"E"	"	h'd.	+	"	+	"	"	+	"G" ser. dil.	=0.7
"G"	"	"	+	"	+	"	"	+	"E" " "	=4.1

It is evident that all mixtures depending on "E" serum heated for phagocytosis show no so-called activation. Hence the cause of the negative results with "E" serum must be attributed to some peculiarity in the thermostable opsonic element in this serum.

Sera from different individuals were now tested to ascertain the relative frequency of this phenomenon of "inactivability" and in nine of twelve individuals results were obtained similar to those with serum "E."

To find whether or not "E" serum would act in the same way toward other organisms as toward pneumococcus, it was tested with

one strain each of *Strept. pyogenes* and *Staph. albus*. The results are shown in Table 5.

TABLE 5.

Mixtures	Phagocytosis *Strept.*	*Staph.*
"E" ser. h'd. + leucocytes + bact. susp. + NaCl sol.	=2.5	1.5
" " dil. + " + " " + " "	=2.3	5.4
" " " + " + " " + "E" ser. h'd.	=4.0	7.4
" " nor. + " + " " + NaCl sol.	=6.5	9.5
"G" " h'd. + " + " " + " "	=3.1	1.5
" " " + " + " " + "E" ser. dil.	=7.7	8.4
" " nor. + " + " " + NaCl sol.	=9.5	11.3

From the results given, it is evident that the behavior of "E" serum toward streptococcus is like that toward pneumococcus. With staphylococcus, on the other hand, there is apparently some degree of reactivation, though not so great as in the case of serum "G."

From the work of Dean, Neufeld and Bickel, and Cowie and Chapin, it would appear that normal opsonin may consist of thermostable and thermolabile elements, similar to the amboceptor and complement constituting bacterial antibodies. Neufeld,[1] indeed, views the process of opsonification as one of incipient lysis, basing his view chiefly on the results observed by himself and Bickel. However, he notes that at times concentrated normal serum, even after heating, favors phagocytosis of certain organisms; this phenomenon he does not attempt to explain.

Levaditi,[2] as well as Muir and Martin,[3] hold the view that opsonins in normal sera are, at least for the greater part, complements. This view Levaditi bases on the following facts: (1) like complement, opsonin is removed from rabbit serum by various micro-organisms and by cellular débris; (2) the serum of rabbits, injected with guinea-pig serum, neutralizes both the complement and opsonin of guinea-pig serum, the neutralization being specific; (3) phosphorous poisoning reduces both complement and opsonin; (4) aqueous humor contains neither complement nor opsonin. None of these facts is incompatible with the view that the complement-like constituent of opsonin, and complement, are similar in behavior, and the apparent removal of opsonin results from removal of its complement-like constituent only.

[1] *Arb. a. d. kais. Gesundh.*, 1907, 27, p. 414.

[2] *Compt. rend. Soc. de Biol.*, 1907, 67, p. 683.

[3] *Brit. Med. Jour.*, 1906, 2, p. 1783; *Proc. Roy. Soc.*, 1907, 79, p. 187.

SUMMARY.

The results of Dean and of Cowie and Chapin to the effect that diluted fresh normal serum may increase greatly the opsonic power of heated serum are confirmed. At the same time a large proportion (75 per cent) of the different sera tested were found to contain inactivable thermostable elements at least with respect to the pneumococcus. In the case of one serum the thermostable element proved inactivable with respect to pneumococcus and streptococcus and activable in a small degree with respect to staphylococcus.